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Pharmaceutical with stabilised granulocytes colony stimulating factor - contains surfactant, saccharide, protein or high mol. wt. cpd. as stabiliser
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DE 3723781	A	19880121	DE 3723781	A	19870717	198804	B
GB 2193631	A	19880217	GB 8716904	A	19870717	198807	
FR 2601591	A	19880122	FR 8710156	A	19870717	198811	
NL 8701640	A	19880216	NL 871640	A	19870713	198811	
AU 8775665	A	19880121				198812	
NO 8702966	A	19880215				198812	
SE 8702907	A	19880119				198812	
DK 8703683	A	19880119				198815	
ZA 8705268	A	19880127	ZA 875268	A	19870717	198818	
HU 44941	T	19880530				198825	
JP 63146826	A	19880618	JP 87178031	A	19870716	198830	
JP 63146827	A	19880618	JP 87178032	A	19870716	198830	
JP 63146828	A	19880618	JP 87178034	A	19870716	198830	
JP 63152326	A	19880624	JP 87178033	A	19870716	198831	
PT 85343	A	19880729				198835	
BE 1000253	A	19880927	BE 88787	A	19880927	198840	
CN 8704963	A	19880224				198915	
CH 671157	A	19890815				198937	
ES 2010226	A	19891101	ES 872106	A	19870717	199004	
GB 2193631	B	19901121				199047	
IT 1218927	B	19900424				199211	
CA 1297007	C	19920310				199216	
NO 171828	B	19930201	NO 872966	A	19870716	199310	
IL 83220	A	19930131	IL 83220	A	19870717	199311	
KR 9304597	B1	19930601	KR 877804	A	19870718	199423	
RU 2025120	C1	19941230	SU 4203033	A	19870717	199531	
SE 503312	C2	19960513	SE 872907	A	19870717	199625	
AT 8701775	A	19960815	AT 871775	A	19870714	199637	
DK 171308	B	19960902	DK 873683	A	19870715	199641	
JP 2577742	B2	19970205	JP 87178031	A	19870716	199710	
JP 2577743	B2	19970205	JP 87178032	A	19870716	199710	
AT 402259	B	19970215	AT 871775	A	19870714	199713	
JP 2629000	B2	19970709	JP 87178033	A	19870716	199732	
NL 192917	B	19980105	NL 871640	A	19870713	199807	
PH 28830	A	19950314	PH 35555	A	19870717	199844	
DE 3723781	C2	19990902	DE 3723781	A	19870717	199939	

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NO 171828	B			A61K-037/02	Previous Publ. patent NO 8702966
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DK 171308	B			A61K-038/18	Previous Publ. patent DK 8703683
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JP 2577743	B2		6	A61K-038/00	Previous Publ. patent JP 63146827
AT 402259	B			A61K-038/19	Previous Publ. patent AT 8701775
JP 2629000	B2		5	A61K-038/00	Previous Publ. patent JP 63152326
NL 192917	B		11	A61K-047/00	
IL 83220	A			A61K-037/02	
KR 9304597	B1			A61K-037/02	
SE 503312	C2			A61K-038/19	
AT 8701775	A			A61K-038/19	
PH 28830	A			A61K-031/715	
DE 3723781	C2			A61K-038/16	

Abstract (Basic): DE 3723781 A

A pharmaceutical contains stabilised G-CSF (granulocytes colony stimulating factor) as active ingredient and at least one surfactant, saccharide, protein or a high mol. wt. cpd. Pref. the amt. of surfactant or saccharide is 1-10000 pts. wt. Pref. pt. wt. G-CSF. Pref. the surfactant is non-ionic (esp. a sorbitan ester glycerine ester, poly-glycerine ester, polyoxyethylene sorbital ester, polyoxyethylene-glycerine ester or polyethylene glycol ester of an aliphatic acid, polyoxyethylene polyoxypropylene alkyl ether, a hardened polyoxyethylated castor oil, a polyoxyethylated bees wax deriv. a polyoxyethylene lanoline deriv. or an aliphatic polyoxyethylene acid aride), anionic (esp. an alkylsulphate or alkylsulpho succinyl ester salt) or natural (esp. lecithin, sphingophospholipid or an ester of an aliphatic acid with sucrose.

USE/ADVANTAGE - G-CSF can be used to treat various infectious diseases, but is unstable and highly sensitive to changes in the environment e.g. temp., humidity, oxygen or UV light. The invention stabilises the G-CSF and protects it completely against loss of activity.

Title Terms: PHARMACEUTICAL; STABILISED; GRANULOCYTE; COLONY; STIMULATING; FACTOR; CONTAIN; SURFACTANT; SACCHARIDE; PROTEIN; HIGH; MOLECULAR; WEIGHT ; COMPOUND; STABILISED

Derwent Class: A96; B04

International Patent Class (Main): A61K-009/14; A61K-031/715; A61K-037/02; A61K-038/00; A61K-038/16; A61K-038/18; A61K-038/19; A61K-047/00

International Patent Class (Additional): A61K-009/08; A61K-031/70; A61K-035/00; A61K-037/00; A61K-037/04; A61K-037/12; A61K-037/43; A61K-038/17; A61K-045/05; A61K-047/10; A61K-047/14; A61K-047/20; A61K-047/24; A61K-047/26; A61K-047/32; A61K-047/34; A61K-047/36; A61K-047/42; C12N-000/00

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(54) Stable granulocyte colony stimulating factor composition

(57) A stable granulocyte colony stimulating factor-containing pharmaceutical preparation contains, in addition to the active agent, at least one substance selected from a pharmaceutically acceptable surfactant, saccharide, protein and high-molecular weight compound.

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SPECIFICATION

Stable pharmaceutical preparation conteining granulocyte colony stimulating factor and procass for producing the sama

The present invention reletes to a phermeceutical preperation containing a granulocyte colony stimulating factor. In particular, the present invention relates to e stabilized pharmeceutical preparation conteining a grenulocyte colony stimulating factor that is protected against loss or inactivation of the ective component (i.e., granulocyta colony stimulating factor) due to adsorption on the well of e container in which the preperation is put, or to association, polymerization or oxidation of said component.

Chemotherapy has been undertaken es one method for treating e variety of infectious diseases but it has recently been found that chemotherapy causes some serious clinicel problems such as the generation of drug-resistant organisms, change of ceusative organisms, and high side effects. In order to avoid these problems essociated with chemotherapy involving the use of therapeutic egents such es entibiotics and bectericides, attempts ere being made to use a substance that activates the prophylectic capebilities of the host of an infection-causing organism end thereby providing a complete solution to the aforementioned problems of chemotherapy. Of the various prophylactic capabilities of the host, the phagocytic bactericidel action of leucocytes is believed to cause the strongest influence in the initial period of bacteriel infection and it is therefore assumed to be important to enhance the infection protecting capabilities of the host by promoting the growth of neutrophiles and their differentiation into the mature state. A granulocyte colony stimulating factor (G-CSF) is one of the very useful substances thet exhibit such actions and the same assignee of the present invention previously filed a patent epplication on en infection protecting agent using G-CSF (Japanese Patent Application No. 23777/1985).

As mentioned above, chemotherapy es currently precticed involves various unavoidable problems end intensive efforts are being made to usa e drug substence that is cepable of ectivating the prophylactic functions of the host or the person who has been infected.

Needless to say, G-CSF displeys by itself the ebility to ectivate the prophylactic functions of the host and it has also been found that G-CSF exhibits greater therapeutic effects in clinical applications if it is used in combination with e substance that activates the prophylactic capabilities of the host.

G-CSF is used in a very small amount end e pharmaceutical preparation containing 0.1—500 μg (preferably 5—50 μg) of G-CSF is usually edminlstered at a dose rate of 1—7 tlmes e week per adult. However, G-CSF has e tendency to be adsorbed on the wall of its conteiner such es an empule for injection or a syringe. Therefore, if the drug is used as an injection in such e form as an aqueous solution, it will be adsorbed on the wall of its container such as an empule or a syringe. This either results in the failure of G-CSF to fully exhibit its ectivity as e pharmaceutical agent or necessitetes the incorporation of G-CSF in e more-then-necessary amount meking allowence for its possible loss due to adsorption.

In addition, G-CSF is labile and highly susceptible to environmental factors such es temperature, humidity, oxygen and ultraviolet rays. 8y the agency of such factors, G-CSF undergoes physical or chemical changes such as association, polymerization and oxidation and suffers e great loss in activity. These phenomenon make it difficult to ensure complete accomplishment of a therapeutic act by edministering e very small amount of G-CSF in a very exact manner.

It is therefore necessary to develop a stable pharmaceutical preparation of G-CSF that is fully protected against a drop in the activity of its effective component. This is the principal object of the present invention which provides e steble pharmaceutical preparation of G-CSF.

The present inventors conducted intensive studies in order to enhence the stability of a G-CSF containing phermaceutical preparation and found that this object can effectively be atteined by eddition of e pharmaceutically acceptable surfactant, saccharide, protein or high-moleculer weight compound.

Therefore, the stable G-CSF containing phermaceutical preperetion of the present invention is characterized by containing both G-CSF and at least one substance selected from the group of a pharmaceutically acceptable surfactant, saccheride, protein end high-molecular weight compound.

The G-CSF to be contained in the pharmaceutical preparetion of the present invention can be obtained by eny of the methods such es those described in the specifications of Japenese Patent Applicetion Nos. 153273/1984, 269455/1985, 269456/1985, 270838/1985 end 270839/1985. For exemple, a human G-CSF can be prepered either by cultivating e cell strain (CNCM Accession Number I-315 or I-483) collected from tumor cells of patients with oral cavity cancer, or by expressing e recombinent DNA (which has been prepared by the egency of a human G-CSF encoding gene) in an appropriate host cell (e.g. *E. coli*, C 127 cell or ovary cells of a Chinese hemster).

Any humen G-CSF that has been purified to hlgh degree may be employed as the G-CSF to be conteined in the phermaceutical preparetion of the present invention. Preferable human G-CSFs

ere ones obteined by isolation from the supernatant of the culture of a human G-CSF producing cell, end a polypeptide or glycoprotein heving the humen G-CSF activity that is obtelned by transforming a host with a recombinant vector having incorporated therein a gene coding for a polypaptide having the human G-CSF ectivity.

Two particularly preferable axemples of humen G-CSF are shown below:

(1) humen G-CSF heving the following physicochemical properties:

i) moleculer weight: ebout 19,000 ± 1,000 es measured by electrophoresis through e sodium dodecylsulfate—polyacrylamide gel;

ii) isoelectric point: heving et least one of the three isoelectric points, pl = 5.5 ± 0.1, pl = 5.8 ± 0.1, end pl = 6.1 ± 0.1;

iii) ultraviolet ebsorption: having a meximum absorption at 280 nm end a minimum absorption et 250 nm;

iv) amino acid sequence of the 21 residues from N terminus: H_2N-Thr-Pro-Leu-Gly-Pro-Ala-Ser-Ser-Leu-Pro-Gln-Ser-Phe-Leu-Leu-Lys-Cys-Leu-Glu-Gln-Val

(2) humen G-CSF containing either e polypeptide having the human granulocyte stimulating factor ectivity which is represented by all or part of the amino acid sequence shown below, or a glycoprotein having both said polypeptide end e sugar chain portion:

(Met)$_n$Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro
Gln Ser Phe Leu Leu Lys Cys Leu Glu Gln Val
Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln
Glu Lys Leu (Val Ser Glu)$_m$Cys Ala Thr Tyr Lys
Leu Cys His Pro Glu Glu Leu Val Leu Leu Gly
His Ser Leu Gly Ile Pro Trp Ala Pro Leu Ser
Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly
Cys Leu Ser Gln Leu His Ser Gly Leu Phe Leu
Tyr Gln Gly Leu Leu Gln Ala Leu Glu Gly Ile
Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu
Gln Leu Asp Val Ala Asp Phe Ala Thr Thr Ile
Trp Gln Gln Met Glu Glu Leu Gly Met Ala Pro
Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala
Phe Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly
Val Leu Val Ala Ser His Leu Gln Ser Phe Leu
Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala
Gln Pro

(provided that m is 0 or 1; and n is 0 or 1).

For details of the method for preparing thase two types of G-CSF, see the specification of Japanesa Patent Application Nos. 153273/1984, 269455/1985, 269456/1985, 270838/1985 end 270839/1985, ell having been filed by the essignee of the present invention.

Another method that can be employed consists of performing fusion of e G-CSF producing cell with a self-prolifereting malignent tumor cell and cultivating the resulting hybridoma in tha presence or absence of mytogen.

The human G-CSF contalning solution obtained may be stored in e frozen state after being further purified and concentrated, as required, by eny known technique. Alternatively, the solu-

tion may be stored after being dehydrated by such means as freaze-drying.

All of the human G-CSFs thus prepared can be processed as specified by the present invention in order to attain stabla G-CSF containing pharmaceutical preparations.

Typical examples of tha surfactant that is used to attain the stabla G-CSF containing pharmaceutical praparation of tha present invention are listed balow: nonionic surfactants with HLB of 6—18 such as sorbitan aliphatic acid esters (e.g. sorbitan monocaprylata, sorbitan monoleurata and sorbitan monopalmitata), glycerin aliphatic acid astars (a.g. glycerin monocaprylate, glycerin monomyristate, and glycarin monosteerate), polyglycerin allphatic ecid esters (e.g. deceglycaryl monostaarate, dacaglycaryl distearata and decaglyceryl monolinoleate), polyoxyethylena sorbitan aliphatic ecid esters (a.g. polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate, polyoxyethylena sorbitan monostearata, polyoxyethylene sorbiten monopelmitata, polyoxyethylene sorbitan trioleete, end polyoxyethylena sorbitan tristearete), polyoxyathylene sorbitol eliphatic acid astars (e.g. polyoxyathylena sorbitol tetrastearate and polyoxyethylane sorbitol tetraoleate), polyethylene glycerin aliphatic ecid estars (a.g. polyoxyathylene glyceryl monostearate), polyethylane glycol eliphatic acid esters (a.g. polyathylene glycol distearate), polyoxyethylena elkyl ethers (e.g. polyoxyethylene lauryl ether), polyoxyethylana polyoxypropylene alkyl ethers (e.g. polyoxyethylene polyoxypropylene glycol ethar, polyoxyethylene polyoxypropylene propyl ether, end polyoxyethylene polyoxypropylana cetyl ather), polyoxyethylena alkylphenyl ethers (e.g. polyoxyethylane nonylphenyl ether), polyoxyethylated castor oil, polyoxyethylated hardened castor oil (polyoxyethyleted hydrogenated castor oil), polyoxyathylated beeswax derivatives (e.g. polyoxyethyleted sorbitol beeswax), polyoxyethylene lanolin derivatives (e.g. polyoxyethylene lanolin), and polyoxyethylena aliphatic acid amides (a.g. polyathylane stearic acid amide); nonionic surfactants such as alkyl sulfuric ecid selts heving e C_{10}—C_{18} alkyl group (a.g. sodium cetyl sulfata, sodium lauryl sulfate and sodium olayl sulfate), polyoxyethylena alkyl ether sulfuric ecid salts wherein the average molar number of ethylene oxide addition is 2—4 and the alkyl group has 10—18 carbon atoms (a.g. polyoxyethylena sodium lauryl sulfata), selts of alkyl sulfosuccinate esters wherein the alkyl group has 8 -18 carbon atoms (a.g. sodium lauryl sulfosuccinata ester); and natural surfactants such as lecithin, glycerophospholipid, sphingophospholipid (e.g. sphingomyelin), and sucrose aliphatic acid esters wherein tha aliphatic acid has 12—18 cerbon atoms. These surfactants may of course be used elther independently or in admixture.

The surfactants listed above are preferably used in amounts of 1—10,000 parts by weight par part by weight of G-CSF.

Tha saccharida to be used in making tha stable G-CSF containing pharmaceuticel preparation of tha present invention may ba selected from emong monosaccherides, oligosacchanidas, and polyseccharidas, as well as phosphate estars and nucleotide derivatives thereof so long es they are pharmaceutically acceptabla. Typical axamplas are listed below: trivalent and higher sugar alcohols such es glycerin, erythritol, arabitol, xylitol, sorbitol, end mannitol; acidic sugars such as glucuronic ecid, iduronic ecid, neuraminic ecid, gelecturonic acid, gluconic acid, mannuronic acid, ketoglycolic ecid, ketogalactonic acid end ketogulonic acid; hyeluronic acid and salts thereof, chondroitin sulfata and salts theraof, heparin, inulin, chitin end derivatlvas thereof, chitosan and derivetives thereof, dextrin, dextran with en average molecular weights of 5,000 - 150,000, and alginic acid and salts thereof. All of these saccharides may be usad with advantage either independently or in admixtura.

The saccharides listed above ere preferably used in amounts of 1—10,000 parts by weight per part by weight of G-CSF.

Typical examples of the protein to ba used in making tha steble G-CSF containing pharmacautical preparation of the present invention includa humen serum albumin, human serum globulin, gelatin, ecid-treated gelatin (averaga mol. wt. = 7,000—100,000), alkali-treated gelatin (average mol. wt. = 7,000—100,000), and collagan. Needless to say, these proteins may be usad either independently or in admixture.

The protains listed above ara preferably usad in amounts of 1—20,000 parts by weight per part by weight of G-CSF.

Typical axemples of the high-molecular weigh compound to be used in making tha stabla G-CSF containing pharmaceutical preparation of tha present Invention include: natural polymers such as hydroxypropyl cellulose, hydroxymethyl cellulose, sodium carboxymethyl cellulosa, and hydroxyethyl callulose; and synthetic polymers such as polyethylene glycol (mol. wt. = 300—6,000), polyvinyl alcohol (mol. wt. = 20,000—100,000), end polyvinylpyrrolidona (mol. wt. = 20,000—100,000). Naedlass to say, these high-molecular weight compounds may be used eithar alone or in combination.

The high-molecular weight compounds listed abova ara desirably used in amounts of 1—20,000 parts by weight per part by weight of G-CSF.

In addition to tha surfactant, saccharida, protein or high-molecular weight compound descnbad abova, at laast one member selected from tha group consisting of en amino acid, e sulfureous reducing agent and an antioxident may elso be incorporated in making the G-CSF conteining pharmaceutical preparetion of tha present invention. Illustrativa emino acids include glycine,

threonine, tryptophan, lysine, hydroxylysine, histidine, erginine, cysteine, cystine, end methionine. Illustrative sulfureous reducing agents include: N-acetylcysteine, N-ecetylhomocysteine, thioctic ecid, thiodiglycol, thioethanolamine, thioglycerol, thiosorbitol, thioglycolic acid and salts thereof, sodium thiosulfate, sodium hydrogensulfite, sodium pyrosulfite, sodium sulfite, thiolactic ecid, dithiothreitol, glutethione, and a mild sulfureous reducing egent having a sulfhydryl group such as a C_1—C_7 thioalkenoic acid. Illustrative enti-oxidants include erythorbic acid, dibutylhydroxytoluene, butylhydroxyenisole, dl-α-tocopherol, tocopherol acetete, L-ascorbic acid end selts theraof, L-ascorbic acid palmitate, L-ascorbic acid stearete, triemyl gallate, propyl gallete end chelating egents such as disodium ethylenedieminetetraacetate (EDTA), sodium pyrophosphate end sodium metephosphata.

The ebove-listed amino acids, sulfuraous reducing egents and antioxidants or mixtures thereof are preferably used in amounts of 1—10,000 parts by weight per pert by weight of G-CSF.

For the purpose of formuleting the stable G-CSF conteining preparation of the present invention in e suitable dosage form, one or more the following egents may be incorporated: e diluent, a solubilizing aid, an isotonic agent, an excipient, a pH modifier, a soothing agent, and a buffer.

The stabilized G-CSF pharmaceutical preparation of the present invention may be formuleted either for oral administration or for parenterel administration such as by injection appiled in various ways, and a variety of dosage forms may be employed depending upon the specific mode of edministration. Typical dosage forms include: those intended for oral administration such as teblets, pills, capsules, granules and suspensions; solutions, suspensions and freeze-dried preparations principally intended for intravenous injection, intremuscuier injection, subcutaneous injection and intracutaneous injection; and those intended for trensmucosal administration such as rectel suppositories, nasal drugs, and vaginal suppositories.

According to the present invention, at leest one substance selected from the group consisting of a surfactant, e seccharide, a protein or e high-moleculer weight compound is edded to a G-CSF containing pharmaceutical preparation so that it is prevented from being adsorbed on the wall of its container or e syringe while at the same time, it remains stable over a prolonged period of time.

The detailed mechanism by which the substances mentioned above stabilized G-CSF or prevent it from being edsorbed is yet to be clarified. In the presence of a surfactant, the surface of G-CSF which is a hydrophobic protein would be covered with the surfactant to become solubilized so that the G-CSF present in a trace emount is effectively prevented from being adsorbed on the wall of its container or e syringe. A saccharide or hydrophilic high-molecular weight compound would form a hydrated leyer between G-CSF end the adsorptive surface of the well of its conteiner or e syringe, thereby preventing adsorption of G-CSF in an effective manner. A protein would compete with G-CSF for edsorption on the well of its container or a syringe, thereby effectively inhibiting adsorption of G-CSF.

Besides the prevention of G-CSF adsorption, the substances mentioned above would elso contribute to the prevention of associetion or polymerization of the molecules of G-CSF. In the presence of e surfactant, saccharide, protein or high-molecular weight compound, the individual molecules of G-CSF are dispersed in these substances and the interaction between the G-CSF molecules is sufficiently reduced to cause a significent decrease in the probability of their association or polymerization. In addition, these substances would retard the autoxidation of G-CSF thet is accelerated under high temperature or humidity or prevent G-CSF from being associated or polymerized es a result of its autoxidation. These effects of retarding autoxidation of G-CSF or preventing it from being associated or polymerized would be further enhanced by addition of an amino acid, a sulfureous reducing egent or an entioxident.

The problems described above are particularly noticeable in solutions for injection and in suspensions but they elso occur during the process of formulating G-CSF in other dosage forms such as tablets. The eddition of surfactants, seccharides, proteins or high-molecular weight compounds is also effective in this latter case.

Through the addition of at least one substence selected from the group consisting of a surfectant, saccheride, protein and a high-molecular weight compound, G-CSF is highly stabilized and meintains its activity for a prolonged period of time, as will be demonstreted in the exemples that follow. To attein these results, the amount of eech of these substances, in particular its lower limit, is critical and the following ranges are desirable: 1—10,000 perts by weight of surfectant, 1—10,000 parts by weight of saccharide, 1—20,000 parts by weight of protein, and 1—20,000 parts by weight of high-molecular weight compound, per 1 pert by weight of G-CSF.

According to the present invention, a surfactant, e saccharide, a protein and/or e high-molecular weight compound is used in a specified concentration and this is effective not only in preventing G-CSF from being adsorbed on the wall of its container or a syringe but elso in enhancing the stability of a G-CSF conteining phermaceuticel preparation. As a result, it becomes possible to ensure the administretion of a small but highly precise dose of G-CSF to patients; since G-CSF is costly, its efficient utilizetion will leed to lower costs for the production of G-CSF

containing phermeceutical preparations.

The following examples are provided for the purpose of further illustrating the present invention but are In no sense to be taken es limiting. In these examples, the residual ectivity of G-CSF was determined by one of the following methods.

(a) *Soft egar method using mouse bone merrow cells*:

A horse serum (0.4 ml), 0.1 ml of the semple, 0.1 ml of e C3H/He (female) mouse bone merrow cell suspension (0.5 -1×10^5 nuclear cells), and 0.4 ml of a modified McCoy's 5A culture solution conteining 0.75% of agar were mixed, poured into e plastic dish for tissue culture (35 mm$^\phi$), coagulated, and cultured for 5 days et 37°C in 5% CO_2/95% eir end at 100% humidity. The number of colonies formed wes counted (one colony consisting of et least 50 cells) and the activity was determined with one unit being the ectivity for forming one colony.

The modified McCoy's 5A culture solution used in the method (a) was prepered by the following procedures.

Modified McCoy's 5A culture solution (double concentration)

Twelve grams of McCoy's 5A culture solution (Gibco), 2.55 g of MEM amino ecid-vitamin medium (Nissui Seiyeku Co., Ltd.), 2.18 g of sodium bicerbonete end 50,000 units of potessium penicillin G were dissolved twice in 500 ml of distilled water and the solution was aseptlcelly filtered through a Millipore filter (0.22 μm).

(b) *Reverse-phase high-performance liquid chromatography*:

Using e reverse-phase C8 column (4.6 mm × 300 mm; 5 μm) and en n-propanol/trifluoroecetic acid mixture as e mobile phese, the residuel ectivity of G-CSF (injected in an emount equivalent to 1 μg) was determined under the following gradient conditions:

Time (sec)	Solvent (A)	Solvent (B)	Gradient
0	100%	0%	linear
15	0%	100%	linear
25	100%	0%	

Solvent (A): 30% n-propanol and 0.1% trifluoroecetic ecid
Solvent (B): 60% n-propanol end 0.1% trifluoroacetic acid

Detection was conducted et e wavelength of 210 nm and the percentage of the residual G-CSF activity was calculated by the following formula:

$$\text{Residual G-CSF activity (\%)} = \frac{\text{the residual amount of G-CSF after the lapse of a given time}}{\text{the initial amount of G-CSF}} \times 100$$

The residual emount of G-CSF as determined by this method correlated very well with the result attained in measurement by the soft eger method (a) using mouse bone marrow cells.

Example 1

To 5 μg of G-CSF, one of the stabilizing agents listed in Table 1 wes edded end the mixture was aseptically dissolved in e 20 mM buffer solution (contalning 100 mM sodium chloride; pH 7.4) to make a pharmaceutical preperation containing 5 μg of G-CSF per ml, which was then freezer-dried. The time-dependent change in G-CSF actlvity was measured by method (e) end the results are shown in Teble 1. The term "ectivity (%)" in the teble represents the residual activity of G-CSF relative to the initiel unit end is defined by the following formula:

$$\text{Activity (\%)} = \frac{\text{activity unit after the lapse of a given time}}{\text{initial activity unit}} \times 100$$

Freeze-drying was conducted by the following procedures:

The G-CSF solution conteining e stebilizing egent was put into a sterile sulfe-treated glass viel, frozen at −40°C or below for 4 hours, subjected to primary drying by heating from −40°C to 0°C over e period of 48 hours with the pressure increased from 0.03 to 0.1 torr, then to secondary during by heating from 0°C to 20°C for e period of 12 hours with the pressure

increased from 0.03 to 0.08 torr; thereefter, the interior of the viel was filled with a sterile dry nitrogen gas to ettein an etmospheric pressure and the vial wes plugged with a freeze-drying rubber stopper, then sealed with an aluminum cap.

Table 1

Stabilizing agent	Amount (parts by weight)	Activity (%)	
		After storage at 4°C for 6 months	After storage at 37°C for 1 month
xylitol	10,000	92	86
mannitol	10,000	91	85
glucuronic acid	10,000	86	82
hyaluronic acid	2,000	92	89
dextran (m.w. 40,000)	2,000	95	90
heparin	5,000	85	80
chitosan	2,000	93	91
alginic acid	2,000	90	90
human serum albumin	1,000	98	99
human serum globulin	1,000	98	95
acid-treated gelatin	2,000	97	95
alkali-treated gelatin	1,000	99	96
collagen	2,000	95	90
polyethylene glycol (m.w. 4,000)	10,000	94	90
hydroxypropyl cellulose	1,000	98	94
sodium carboxymethyl cellulose	1,000	88	80
hydroxymethyl cellulose	5,000	92	90
polyvinyl alcohol (m.w. 50,000)	2,000	96	95
polyvinylpyrrolidone (m.w. 50,000)	2,000	95	94
human serum albumin mannitol cysteine	2,000 2,000 100	100	97

Table 1 (cont'd)

Stabilizing agent	Amount (parts by weight)	Activity (%)	
		After storage at 4°C for 6 months	After storage at 37°C for 1 month
human serum albumin polyoxyethylene sorbitan monolaurate mannitol	2,000 100 2,000	99	96
human serum albumin hydroxypropyl cellulose dextran (m.w. 40,000)	2,000 500 2,000	98	92
polyoxyethylene sorbitan monolaurate sorbitol	100 2,000	98	96
polyoxyethylated hardened castor oil dextran (m.w. 40,000)	100 2,000	94	92
not added	-	74	58

Example 2

To 10 μg of G-CSF, one of the stabilizing agents listed in Table 2 was added and the mixture was aseptically dissolved in a 20 mM phosphate buffer solution (containing 100 mM sodium chloride; pH, 7.4) to make a pharmaceutical preparation containing 10 μg of G-CSF per ml. The preparation wes asaptically charged into e sulfa-treated glass vial end sealed to make a G-CSF solution. The tima-dependent change in the activity of G-CSF in this solution was measured by the same method as used in Example 1 and the results are shown in Table 2.

Table 2

Stabilizing agent	Amount (parts by weight)	Activity (%)		
		After storage at 4°C for 7 days	After storage at 4°C for 2 months	After storage at RT for 1 month
mannitol	5,000	91	87	82
hyaluronic acid	2,000	93	87	70
dextran (m.w. 40,000)	2,000	96	95	85
glycerin	10,000	90	90	88
neuraminic acid	5,000	93	91	84
chitin	2,000	95	92	86
dextrin	2,000	90	92	87
human serum albumin	1,000	99	95	92
human serum globulin	1,000	98	94	90
acid-treated gelatin	2,000	97	96	87
alkali-treated gelatin	500	99	95	92
collagen	2,000	99	94	88
polyethylene glycol (m.w. 4,000)	10,000	94	89	90
hydroxypropyl cellulose	2,000	98	95	92
sodium carboxymethyl cellulose	2,000	92	91	80
hydroxyethyl cellulose	4,000	92	94	90
polyvinyl alcohol (m.w. 50,000)	4,000	97	93	90
polyvinylpyrrolidone (m.w. 50,000)	4,000	95	95	92
sorbitan monolaurate	400	97	96	95
polyoxyethylene sorbitan monolaurate	400	100	96	94

Table 2 (cont'd)

Stabilizing agent	Amount (parts by weight)	Activity (%)		
		After storage at 4°C for 7 days	After storage at 4°C for 2 months	After storage at RT for 1 month
polyoxyethylene sorbitan monostearate	400	98	97	94
polyoxyethylene polyoxypropylene glycol ether	400	100	94	93
polyoxyethylated hardened castor oil	400	99	98	90
sodium lauryl sulfate	2,000	97	93	87
lecithin	2,000	97	94	90
human serum albumin mannitol cysteine	2,000 2,000 100	100	99	97
human serum albumin polyoxyethylene sorbitan monolaurate mannitol	2,000 100 2,000	99	97	95
human serum albumin hydroxypropyl cellulose dextran (m.w. 40,000)	1,000 500 2,000	99	97	95
polyoxyethylene sorbitan monopalmitate sorbitol	100 2,000	96	96	93
polyoxyethylated hardened castor oil dextran (m.w. 40,000)	100 2,000	95	92	92
not added	-	72	61	47

Example 3

To 10 μg of G-CSF, one of the stabilizing agents listed in Table 3 was added and the mixture was aseptically dissolved in a 20 mM phosphate buffer solution (containing 100 mM sodium chloride; pH, 7.4) to make a pharmaceutical preparation containing 10 μg of G-CSF per ml. One milliliter of the preparation was charged into a sulfa-treated silicone-coated glass vial and left at 4°C. The effectiveness of each stabilizing agent in preventing G-CSF adsorption was evaluated by measuring the residual activity of G-CSF in the solution after 0.5, 2 and 24 hours. The measurement was conducted by method (b) using reverse-phase high-performance liquid chromatography. The results are shown in Table 3.

Table 3

Stabilizing agent	Amount (parts by weight)	Residual activity (%)			
		initial	0.5 h	2 h	24 h
monnitol	5,000	100	93	90	91
hyaluronic acid	2,000	100	97	92	92
dextran (m.w. 40,000)	2,000	100	98	95	96
glycerin	10,000	100	94	91	90
heparin	2,000	100	92	90	90
glucuronic acid	5,000	100	96	90	91
ketoglycolic acid	5,000	100	92	88	90
human serum albumin	1,000	100	100	101	99
human serum globulin	1,000	100	98	100	98
alkali-treated gelatin	500	100	99	98	99
acid-treated gelatin	2,000	100	99	97	97
collagen	2,000	100	100	98	99
polyethylene glycol (m.w. 4,000)	10,000	100	100	100	99
hydroxypropyl cellulose	2,000	100	100	100	99
sodium carboxymethyl cellulose	2,000	100	98	96	95
hydroxyethyl cellulose	4,000	100	96	93	92
polyvinyl alcohol (m.w. 50,000)	4,000	100	99	100	98
polyvinylpyrrolidone (m.w. 50,000)	4,000	100	98	98	96
sorbitan monocaprylate	400	100	100	100	98
polyoxyethylene sorbitan monostearate	400	100	100	98	100
polyoxyethylated hardened castor oil	400	100	99	101	99

Table 3 (cont'd)

Stabilizing agent	Amount (parts by weight)	Residual activity (%)			
		initial	0.5 h	2 h	24 h
sodium lauryl sulfate	2,000	100	100	99	97
lecithin	2,000	100	99	100	98
human serum albumin mannitol cysteine	2,000 2,000 100	100	100	100	101
human serum albumin polyoxyethylene sorbitan monolaurate mannitol	2,000 100 2,000	100	100	98	99
human serum albumin hydroxypropyl cellulose dextran (m.w. 40,000)	1,000 500 2,000	100	101	99	100
polyoxyethylene sorbitan monolaurate sorbitol	100 2,000	100	100	99	99
polyoxyethylated hardened castor oil dextran (m.w. 40,000)	100 2,000	100	100	98	97
not added	-	100	91	72	73

CLAIMS

1. A stable granulocyte colony stimuleting factor conteining pharmaceuticel preparation thet contains, in addition to the grenulocyte colony stimulating factor present as the effective ingredient, at least one substance selected from the group consisting of a pharmaceutically acceptable surfactant, saccharide, protein and high-moleculer weight compound.

2. A steble granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 which contains the surfactant in en amount of 1—10,000 perts by weight per part by weight of the granulocyte colony stimulating factor.

3. A stable granulocyte colony stimuleting factor conteining phermeceutical preperation according to Claim 1 or 2 wherein seld surfactant is at least one member selected from the group consisting of e nonionic surfactant, en enionic surfactant end e naturel surfectant, the nonionic surfactant being e sorbiten eliphatic acid ester, e glycerin aliphatic acid ester, a polyglycerin aliphatic acid ester, a polyoxyethylene sorbitan eliphatic ecid ester, e polyoxyethylene sorbitol aliphatic ecid ester, e polyoxyethylene glycerin eliphatic acid ester, e polyethylene glycol aliphatic ecid ester, a polyoxyethylene elkyl ether, a polyoxyethylene polyoxypropylene alkyl ether, e polyoxyethylene elkylphenyl ether, a polyoxyethylated hardened castor oil, e polyoxyethylated beeswax derivative, a polyoxyethylene lenolin derivative, or a polyoxyethylene aliphatic acid emide, the anionic surfactant being an alkyl sulfate selt, a polyoxyethylene alkyl ether sulfete selt, or en elkyl sulfosuccinate ester salt, end the netural surfactant beIng lecithin, glycerophospholipid, sphingophospholipid, or a sucrose aliphatic ecid ester.

4. A stable granulocyte colony stimulating factor containing pharmaceutical preparetion according to Claim 1 which conteins the saccharide in en amount of 1—10,000 perts by weight per part by weight of the grenulocyte colony stimuleting fector.

5. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 or 4 wherein said sacchanide is at least one member selected from the group consisting of glycerin, erythritol, arabitol, xylitol, sorbitol, mannitol, glucuronic ecid, iduronic ecid, galacturonic acid, neuraminic ecid, glyconic acid, mannuronic acid, ketoglycolic acid, ketogalactonic acid, ketogulonic acid, hyaluronic ecid and salts thereof, chondroitin sulfete end salts thereof, heparin, inulin, chitin and derivatives thereof, chitosan end derivatives thereof, dextrin, dextran with an everage molecular weight of 5,000 -150,000, and elginic ecid end salts thereof.

6. A steble granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 which contains the protein in an amount of 1—20,000 parts by weight per part by weight of the granulocyte colony stimulating factor.

7. A stable granulocyte colony stimulating factor conteining pharmaceutical preparation according to Claim 1 or 6 wherein said proteln is et least one member selected from the group consisting of human serum albumin, human serum globulin, gelatin, acid- or alkali-treated gelatin with an everage molecular weight of 7,000—100,000, end collagen.

8. A stable granulocyte colony stimulating factor containing pharmaceutical preparation according to Claim 1 which contains the high-molecular weight compound in en amount of 1—20,000 parts by weight per pert by weight of the granulocyte colony stimulating factor.

9. A stable granulocyte colony stimulating factor containing pharmaceutical preparetion according to Claim 1 or 8 wherein said high-molecular weight compound is at least one member selected from the group consisting of hydroxypropyl cellulose, hydroxymethyl cellulose, sodium carboxymethyl cellulose, hydroxyethyl cellulose, polyethylene glycol with a molecular weight of 300—6,000, polyvinyl elcohol with a molecular weight of 20,000—100,000, and polyvinylpyrrolidone with a molecular weight of 20,000—100,000.

10. A process for producing e stable granulocyte colony stimulating factor containing pharmaceutical preperation that contains, in addition to the granulocyte colony stimulating factor present as the effective ingredient, at least one substance selected from the group consisting of a pharmaceutically eccepteble surfactant, saccharide, protain and high-molecular weight compound.

11. A stable granulocyte colony stimulating fector containing phermaceutical preparation substantially as hereinbefore described, with reference to Exemple 1, 2 or 3.

12. A process for producing a stable granulocyte colony stimulating factor containing pharmaceutical preparation substantielly es hereinbefore described, with reference to Example 1, 2 or 3.

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